National Health and Nutrition Examination Survey III
1988-1994

Documentation, Codebook, and Frequencies

ANTIBODY TO CYTOMEGALOVIRUS IGG AND IGM

Survey Years:
1988 to 1994

SAS Export File:
CMV.XPT

December 2005

NHANES III Data Documentation

Laboratory Assessment: Antibody to Cytomegalovirus IgG and IgM (NHANES III Surplus Sera)

Years of Coverage: 1988–1994 **First Published:** December 2005 **Last Revised:** N/A

Component Description

Cytomegalovirus (CMV) antibody testing of stored sera specimens from NHANES III (1988–1994) was conducted to estimate the seroprevalence of CMV infection (CMV IgG) in participants age 6 and above. CMV IgG avidity and IgM were also measured in women age 12-49 years to estimate rates of primary infection.

Eligible Sample

Participants aged 6+ years from of NHANES III with stored sera (N = 21,648).

Description of Laboratory Methodology

CMV specific IgG was measured with an ELISA by Quest International, Inc., Miami FL. Sera with values near the ELISA cutoff (approx 7% of total) were confirmed with a second ELISA assay by bioMerieux, Inc., Durham, NC. When the results from these 2 tests disagreed (approx. 2% of total), an IFA from Bion International, Inc., was used and the result of the IFA was given as the final result.

CMV specific IgM was measured with an ELISA by bioMerieux, Inc., Durham, NC.

CMV IgG avidity was measured with an ELISA by bioMerieux, Inc., Durham, NC. 1= low avidity defined as RFV_INDEX < 0.8 and 2= High avidity defined as RFV_INDEX >= 0.8. IgG avidity is a measure of the overall strength of binding of the IgG antibody to the antigen. The longer since primary infection the stronger the bond will be between IgG and the antigen. Low IgG avidity indicates a recent infection - so this infection is likely primary. High IgG avidity suggests that the primary infection occurred in the past. Those who are IgM positive and have high IgG avidity are likely experiencing a secondary infection (either a reactivation or reinfection).

Laboratory Quality Control and Monitoring

Commercial reagents were used for all CMV testing. All QC procedures recommended by the manufacturers were followed.

Data Processing and Editing

Data was received after all the antibody testing was complete. The data were not edited.
Data Access: All data are publicly available.

Analytic Notes

There are three variables:
CVP_IgG: (1=Positive, 2=Negative, 3= Equivocal)
CVP_IgM: (1= Positive, 2= Negative, 3= Equivocal)
CVP_IGGA: (1=low, 2=high)

References

1. Starr, S.E., and Friedman, H.M. "Human Cytomegalovirus", Chapter 65 in Manual of Clinical Microbiology, fourth edition, edited by E.H. Lennette, A. Balows, W.J. Hausler, Jr., and H.J. Shadomy, American Society for Microbilolgy, pp 711-719. 1985.
2. Peckham, C.S. "Cytomegalovirus in the neonate". Journal of Antimicrobial Chemotherapy. 23:17-21. 1989.
3. Joassin, L., and Reginster, M. "Elimination of nonspecific cytomegalovirus immunoglobulin M activities in the enzyme-linked immunosorbent assay by using anti-human immunoglobulin G". Journal of Clinical Microbiology. 23:576-581. 1986.

Locator Fields

Title: Antibody to Cytomegalovirus IgG and IgM

Contact Number: 1-866-441-NCHS

Years of Content: 1988-1994

First Published: December 2005

Revised: N/A

Access Constraints: None

Use Constraints: None

Geographic Coverage: National

Subject: Antibody to Cytomegalovirus IgG and IgM

Record Source: NHANES III (1988-1994)

Survey Methodology: NHANES III (1988-1994) is a stratified multistage probability sample of the civilian non-institutionalized population of the U.S.

Medium: NHANES Web site; SAS transport files

National Health and Nutrition Examination Survey
Codebook for Data Production (1988-1994)
(NHANES III)

Antibody to Cytomegalovirus IgG and IgM (CMV)
Person Level Data

December /2005

SEQN	Target
	B(6 Yrs. to 150 Yrs.)
Hard Edits	**SAS Label**
	Respondent sequence number
English Text: Respondent sequence number.	
English Instructions:	

CVP_IGG	Target
	B(6 Yrs. to 150 Yrs.)
Hard Edits	**SAS Label**
	Serum CMV IgG

English Text: Serum CMV IgG

English Instructions:

Code or Value	Description	Count	Skip to Item
1	Positive	15477	
2	Negative	6162	
3	Equivocal	9	
.	Missing	0	

CVP_IGM	Target
	B(6 Yrs. to 150 Yrs.)
Hard Edits	**SAS Label**
	Serum CMV IgM

English Text: Serum CMV IgM

English Instructions:

Code or Value	Description	Count	Skip to Item
1	Positive	127	
2	Negative	5873	
3	Equivocal	74	
.	Missing	15574	

CVP_IGGA	Target
	B(6 Yrs. to 150 Yrs.)
Hard Edits	**SAS Label**
	IgG Avidity

English Text: IgG Avidity

English Instructions:

Code or Value	Description	Count	Skip to Item
1	low	55	
2	high	200	
.	Missing	21393	

Division of Laboratory Sciences
Laboratory Protocol

Analyte: bioMerieux CMV IgG
Matrix:
Method: Enzyme-linked fluorescent immunoassay (ELFA)
Method Code:
Branch: REVB

Prepared By: Kay W. Radford _____ _____
author's name | signature | date

Supervisor: Sheila Dollard, PhD _____ _____
supervisor's name | signature | date

Branch Chief: _____ _____
signature | date

Adopted: 8-2003
date

Updated: _____
date

Director's Signature Block:
Reviewed:

_____ _____
signature | date

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signature | date

Modifications/Changes: see Procedure Change Log

Procedure Change Log

Procedure: _bioMerieux CMV IgG_____DLS Method Code: _____

Date	Changes Made	By	Reviewed By (Initials)	Date Reviewed

Laboratory Procedure Manual

Analyte: *bioMerieux CMV IgG*

Matrix:

Method: Enzyme-linked fluorescent immunoassay (ELFA)

Method No:
Revised:

as performed by: *Herpes* Diagnostic Laboratory

contact: Sheila Dollard, PhD

Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

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   c. Instructions for Calibration of Instrument
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ITN-DLS

DLS Method Code:

1. Summary of Test Principle and Clinical Relevance

CMV antigen detects specific antibody in serum. CMV is clinically important in several patient populations.

2. Safety Precautions

Treat all blood products as potentially infectious; wear appropriate personal protection equipment. Dispose of all Solid Phase Receptacle (SPR) and test strips in autoclave pans.

3. Computerization; Data System Management

CMV IgG ELISA use computer programs designed by Biomerieux. All specimen NHANES numbers were scanned by the barcode reader. The VIDAS instrument read specimen reactivity and assigned numeric values.

4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

Specimens arrived frozen from CASPIR and immediately placed at -20°C. Before testing, the specimens were thawed at 4°C. Specimens were kept at 4°C if further testing was needed, or returned to -20°C if testing was complete. Specimens with excessive microbial contamination were not tested.

5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides

Not applicable

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

a. Reagent Preparation

All reagents including standards and controls were from commercial kits.

b. (1) Standards Preparation

Not applicable

c. Preparation of Quality Control Materials

Not applicable

d. Other Materials

e. Instrumentation

VIDAS (**V**itek **I**mmuno**D**iagnostic **A**ssay **S**ystem), model # v5431, bioMerieux Vitek, Inc., 595 Anglum Drive, Hazelwood, Missouri 63042-2395.

7. Calibration and Calibration Verification Procedures

a. Performance Checks for the Assay
Kit controls are included with each run and the kit standards are run every 14 days in duplicate, as per manufacturer instructions.

b. Calibration of Instrument
Calibration kits were run as recommended plus the instruments received preventative maintenance from company service engineers at least annually.

c. Instructions for Calibration of Instrument
Commercial calibration kits were used per manufacturer's instructions annually during preventative maintenance.

8. Procedure Operating Instructions; Calculations; Interpretation of Results
VIDAS is a fully automated closed system that performs every step after 100ul of sample has been added. The computer software from the manufacturer interpreted the results.

9. Reportable Range of Results
<4AU/ml to 400AU/ml

10. Quality Control (QC) Procedures

a. Blind Quality Controls
Not applicable

b. Bench Quality Controls
Not applicable

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria
Testing is repeated if controls and/or standards were out of range.

12. Limitations of Method; Interfering Substances and Conditions
a. A very recent CMV infection (less than three weeks) may have IgG levels <4AU/ml.
b. Rare cross-reactivity can occur with other infectious diseases.

13. Reference Ranges (Normal Values)
Reference ranges of positive and negative controls, and standards were established by the MLE (Master Lot Entry) card for each lot. Controls and standards out of range were flagged by the instrument and therefore the results are void and must be repeated.

14. Critical Call Results ("Panic Values")
Not applicable

15. Specimen Storage and Handling during Testing

Specimens were tested at room temperature and returned to 4°C until further testing. Once all testing was complete, specimens were returned to -20°C.

16. Alternate Methods for Performing Test of Storing Specimens if Test System Fails

Alternate method for performing test, not applicable; however, if test system fails, troubleshoot and the run was repeated. If run fails again, a service call was placed. Specimens will remain at 4°C until testing was complete.

17. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Not applicable

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

Specimens have a barcode which was used for tracking during all laboratory testing. For refrigerator and freezer storage, each specimen was accounted for by box number, and cell number. At completion of testing and analysis, all specimens will be returned to CASPIR CDC storage or destroyed.

DLS Method Code:

References

7. 1. Horodniceanu F., Michelson S. Archives of Virology, 1980,64,287-301.
8. 2. Yolken R.H., Stopa P.J. Journal of Clin. Microbiology, 1980, Vol II, N°6, 546-551.
9. 3. Ahlfors H., Ivarsson S.A., Johnsson T. and Svanberg L. Acta Paediatr Scand 71, 1982, 109-113.

ADDITIONAL SOURCES

ACKNOWLEDGMENTS

Division of Laboratory Sciences
Laboratory Protocol

Analyte: bioMerieux CMV IgM
Matrix:
Method: Enzyme-linked fluorescent immunoassay (ELFA)
Method Code:
Branch: REVB

Prepared By: Kay W. Radford _____ _____
author's name | signature | date

Supervisor: Sheila Dollard, Ph.D _____ _____
supervisor's name | signature | date

Branch Chief: _____ _____
signature | date

Adopted: 8-2003
date

Updated: _____
date

Director's Signature Block:
Reviewed:

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signature | date

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signature | date

Modifications/Changes: see Procedure Change Log

Procedure Change Log

Procedure: _bioMerieux CMV IgM_____DLS Method Code: _____

Date	Changes Made	By	Reviewed By (Initials)	Date Reviewed

Laboratory Procedure Manual

Analyte: *bioMerieux CMV IgM*

Matrix:

Method: Enzyme-linked fluorescent immunoassay (ELFA)

Method No:
Revised:

as performed by: *Herpes* Diagnostic Laboratory

contact: Sheila Dollard, Ph.D

Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

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ITN-DLS

DLS Method Code:

1. Summary of Test Principle and Clinical Relevance

CMV antigen Solid Phase Receptacle (SPR) detects specific antibody in serum. CMV is clinically important in several patient populations and can aid in the detection of CMV primary infection.

2. Safety Precautions

Treat all blood products as potentially infectious; wear appropriate personal protection equipment. Dispose of all Solid Phase Receptacle (SPR) and test strips in autoclave pans.

3. Computerization; Data System Management

CMV IgM ELISA use computer programs designed by bioMerieux. All specimen NHANES number were scanned by the barcode reader. The VIDAS instrument read specimen reactivity and assigned numeric values.

4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

Specimens arrived frozen from CASPIR and were immediately placed @ -20°C. Before testing, the specimens were thawed at 4°C. Specimens were returned to 4°C if further testing needed or returned to -20°C once testing was completed. Specimens with excessive microbial contamination should not be tested.

5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides

Not applicable

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

a. Reagent Preparation

All reagents including standards and controls are from commercial kits.

b. (1) Standards Preparation

Not applicable

c. Preparation of Quality Control Materials

Not applicable

d. Other Materials

e. Instrumentation

VIDAS (**V**itek **I**mmuno**D**iagnostic **A**ssay **S**ystem), model # v5431, bioMerieux Vitek, Inc., 595 Anglum Drive, Hazelwood, Missouri 63042-2395.

7. Calibration and Calibration Verification Procedures

a. Performance Checks for the Assay
Kit controls are included with each run and the kit standards are run every 14 days in duplicate, as per manufacturer instructions.

b. Calibration of Instrument
Calibration kits run as recommended plus the instruments received preventative maintenance from company service engineers at least annually.

c. Instructions for Calibration of Instrument
Commercial calibration kits were used per manufacturer's instructions annually during preventative maintenance.

8. Procedure Operating Instructions; Calculations; Interpretation of Results
VIDAS is a fully automated closed system that performed every step after 100ul of the sample has been added. The computer software from the manufacturer interpreted the results.

9. Reportable Range of Results
<0.70 to ≥0.90

10. Quality Control (QC) Procedures

a. Blind Quality Controls
Not applicable

b. Bench Quality Controls
Not applicable

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria
Testing is repeated if controls and/or standards were out of range.

12. Limitations of Method; Interfering Substances and Conditions
a. IgM responses can vary from patient to patient. A negative result in the VIDAS CMVM assay does not preclude the possibility of recent primary CMV infection.
b. Serum samples with total IgG concentrations of >= 20mg/ml may cause interference in the VIDAS CMVM assay due to incomplete absorption of the IgG.

13. Reference Ranges (Normal Values)
Reference ranges of positive and negative controls, and standards are established by the MLE (Master Lot Entry) card for each lot. Controls and standards out of range are flagged by the instrument and therefore the results are void and must be repeated.

14. Critical Call Results ("Panic Values")
Not applicable

15. Specimen Storage and Handling During Testing

Specimens were tested at room temperature. Once specimens were loaded, they were returned to 4°C until further testing. Once all testing was complete, specimens were returned to -20°C.

16. Alternate Methods for Performing Test of Storing Specimens if Test System Fails

Alternate method for performing test, not applicable; however, if test system fails, troubleshoot and the run was repeated. If run fails again, a service call was placed.

17. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Not applicable

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

Specimens have a barcode which was used for tracking during all laboratory testing. For refrigerator and freezer storage, each specimen was accounted for by box number, and cell number. At completion of testing and analysis, all specimens will be returned to CASPIR CDC storage or destroyed.

References

7. 1. Starr, S.E., and Friedman, H.M. “Human Cytomegalovirus”, Chapter 65 in Meanual of Clinical Microbiology, fourth edition, edited by E.H. Lennette, A. Balows, W.J. Hausler, Jr., and H.J. Shadomy, American Society for Microbilolgy, pp 711-719. 1985.
8. 2. Peckham, C.S. “Cytomegalovirus in the neonate”. Journal of Antimicrobial Chemotherapy. 23:17-21. 1989.
9. 3. Joassin, L., and Reginster, M. “Elimination of nonspecific cytomegalovirus immunoglobulin M activities in the enzyme-linked immunosorbent assay by using anti-human immunoglobulin G”. Journal of Clinical Microbiology. 23:576-581. 1986.

ADDITIONAL SOURCES

ACKNOWLEDGMENTS

Division of Laboratory Sciences
Laboratory Protocol

Analyte: **BION CMV IgG Antibody Test System**
Matrix:
Method: **IFA**
Method Code:
Branch: **REVB**

Prepared By: Kay Radford _____ _____
author's name | signature | date

Supervisor: Sheila Dollard, PhD _____ _____
supervisor's name | signature | date

Branch Chief: _____ _____
signature | date

Adopted: 8-2003
date

Updated: _____
date

Director's Signature Block:
Reviewed:

_____ _____
signature | date

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signature | date

Modifications/Changes: see Procedure Change Log

Procedure Change Log

Procedure: _Bion CMV IgG IFA_____DLS Method Code: _____

Date	Changes Made	By	Reviewed By (Initials)	Date Reviewed

Laboratory Procedure Manual

Analyte: BION CMV IgG Antibody Test System

Matrix:

Method: IFA

Method No:
Revised:

as performed by: Herpesvirus Diagnostic Laboratory

contact: Sheila Dollard, PhD

Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

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ITN-DLS

DLS Method Code:

1. Summary of Test Principle and Clinical Relevance

CMV antigen detects specific antibody in serum. CMV is clinically important in several patient populations.

2. Safety Precautions

Treat all blood products as potentially infectious; wear appropriate personal protection equipment. Care should be taken to avoid splashing and generation of aerosols. Dispose of all waste in the appropriate biohazard container.

3. Computerization; Data System Management

The IFA used no computerization; it was performed manually and results were entered manually.

4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

Specimens arrived frozen from CASPIR and immediately placed @ -20°C. Before testing, the specimens were thawed at 4°C. Upon completion, specimens were returned to 4°C if further testing needed or returned to -20°C. Excessive microbial contamination was the criteria for specimen rejection.

5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides

Stained slides were examined as soon as possible on the same day using a fluorescence microscope. Each slide had a positive and negative control; therefore if the controls fail, the slide was repeated. If an individual well on the slide lacked adequate cells, the results were rejected and the specimen was retested.

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

a. Reagent Preparation

All reagents including controls were from a commercial kit

b. (1) Standards Preparation

Not applicable

c. Preparation of Quality Control Materials

The positive and negative control specimens provided in the kit as well as in-house controls were used.

d. Other Materials

e. Instrumentation

Fluorescent microscope was required to read the slides.

7. Calibration and Calibration Verification Procedures

a. Performance Checks for the Assay

The commercial positive and negative control specimens and in-house negative control specimen were run on each slide. The negative control should have fluorescence value of less than 1+ and appear reddish-orange. If the controls failed, the slide was repeated.

b. Calibration of Instrument

The fluorescent scope receives preventative maintenance and alignment annually.

c. Instructions for Calibration of Instrument

Not applicable

8. Procedure Operating Instructions; Calculations; Interpretation of Results

Examined slides were resulted as negative or assigned a fluorescent intensity grading value: 1+ was very dim fluorescence and interpreted as negative. 2+ was positive but weak fluorescence. 3+ and 4+ were resulted as strongly positive.

9. Reportable Range of Results

Negative up to 4+ intensity

10. Quality Control (QC) Procedures

a. Blind Quality Controls

Not applicable

b. Bench Quality Controls

An in-house CMV IgG negative specimen was added as an internal negative control.

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

If controls fail the results from the slide is void, and must be repeated.

12. Limitations of Method; Interfering Substances and Conditions

A negative test result does not necessarily rule out current or recent infection. Antinuclear antibodies present in serum may interfere with the CMV IFA test.

13. Reference Ranges (Normal Values)

Each control must demonstrate the expected reaction in order to validate the test. If the controls failed, the results were invalid and must be repeated.

14. Critical Call Results (“Panic Values”)

Not applicable

DLS Method Code:

15. Specimen Storage and Handling During Testing

Specimens were removed from 4°, diluted, and then returned to 4°C if further testing was warranted or returned to -20°C if testing was complete.

16. Alternate Methods for Performing Test of Storing Specimens if Test System Fails

Not applicable

17. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Not applicable

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

Specimens were assigned a number by NHANES that was used for identification. For refrigerator and freezer storage, each specimen was accounted for by box number, and cell number. At completion of testing and analysis, all specimens will be returned to CASPIR CDC storage or destroyed.

DLS Method Code:

References

7. 1. Starr, S.E., Cytomegalovirus, Pediatr. Clinical. N. Amer., 26:283-293, 1979.
8. 2. Reynolds, D.W., S. Stagno, C.A. Alford, Laboratory Diagnosis of Cytomegalovirus Infections, in Diagnostic Procedures for Viral, Reckettsial and Chlamydial Infections, Lennett, E.H., N.J. Schmidt (eds). APHA, Washington, D.C., 1979.
9. 3. Hanshaw, J.B., J.A. Dugeon, Congenital Cytomegalovirus. Viral Diseases of the Fetus and Newborn, Saunders, Philadelphia, 1978.

ADDITIONAL SOURCES

ACKNOWLEDGMENTS